amplified *cry1C* fragments recovered from the overlap extension PCR™ and the ligation products used to transform *E. coli* Sure™ cells (Stratagene) to ampicillin resistance using electroporation. Several hundred ampicillin resistant colonies were harvested from Luria plates containing 50 µg/ml ampicillin, suspended in 10 ml of Luria broth containing 50 µg/ml ampicillin, and allowed to grow at 37°C for 1 hour with agitation. Recombinant plasmids from the culture were isolated using the alkaline lysis procedure.

Approximately 0.1 -1.0 microgram of the *cry1C* plasmid preparation was digested with *Nhe*I to linearize plasmid molecules harboring the *Nhe*I site of pEG943. The plasmid preparation was then used to transform the acrystalliferous *B. thuringiensis* strain EG10650 to chloramphenicol resistance using electroporation. Because linear DNAs do not transform *B. thuringiensis* efficiently, this *Nhe*I cleavage step ensures that virtually all of the clones recovered from the transformation encode substitutions at position 220 and lysine at position 219. Individual chloramphenicol resistant colonies were transferred to starch agar or Luria plates containing 3 µg/ml chloramphenicol. To confirm transfer of the *cry1C* plasmids to EG10650, individual clones were inoculated into 3 ml of BHIG containing 3 µg/ml chloramphenicol and grown at 30°C until the cultures were turbid. Plasmid DNAs were isolated from the broth cultures using the alkaline lysis method and the plasmid identities confirmed by restriction enzyme analysis. Cry1C-R148D mutants containing substitutions at S220 were designated Cry1C pr66-1, -2, -3, *etc.*

Amino acid substitutions were also generated at amino acid positions 217, 218, 219, 221, and 222 in Cry1C using this procedure and the following mutagenic oligonucleotide primers:

Position 217: Primer M (SEQ ID NO:65)

5'-CGGGGATTAAATAATNNACCGAAAAGCACGTATCAAGATTGGATAAC-3'

N (16) = 50% C; 50% G

N (17) = 33.3% C; 33.3% G; 33.3% A

Position 218: Primer N (SEQ ID NO:66)

5'-CGGGGATTAAATAATTTANNAAAAAGCACGTATCAAGATTGGATAAC-3'

N (19) = 50% C; 50% G

N (20) = 33.3% C; 33.3% G; 33.3% A

Position 219: Primer O (SEQ ID NO:67)

5'-CGGGGATTAAATAATTTACCGNNAAGCACGTATCAAGATTGGATAAC-3'

N (22) = 50% C; 50% G

N (23) = 33.3% C; 33.3% G; 33.3% A

Position 221: Primer P (SEQ ID NO:68)

5'-GGATTAAATAATTTACCGAAAAGCNNATATCAAGATTGGATAACATATAATCG-3'

N (25) = 50% C; 50% G

N (26) = 33.3% C; 33.3% G; 33.3% A

Position 222: Primer Q (SEQ ID NO:69)

5'-GGATTAAATAATTTACCGAAAAGCACGNNACAAGATTGGATAACATATAATCG-3'

N (28) = 50% C; 50% G

N (29) = 33.3% C; 33.3% G; 33.3% A

Table 15 lists the Cry1C mutants expected from the mutagenesis procedure.

TABLE 15

SUMMARY OF CRY1C-R148D LOOP α6-7 MUTANTS

Amino acid Position	Wild-type amino acid	Primer	Predicted amino acid substitutions	Mutant designation
217	leucine	M	R, E, Q, A, G, P	Cry1C pr67 -1, -2, -3, *etc.*
218	proline	N	R, E, Q, A, G, P	Cry1C pr65 -1, -2, -3, *etc.*
219	lysine	O	R, E, Q, A, G, P	Cry1C pr70 -1, -2, -3, *etc.*
221	threonine	P	R, E, Q, A, G, P	Cry1C pr68 -1, -2, -3, *etc.*
222	tyrosine	Q	R, E, Q, A, G, P	Cry1C pr69 -1, -2, -3, *etc.*

EXAMPLE 11 -- CRY1C-R148D LOOP α5-6 COMBINATORIAL MUTANTS

A similar overlap extension PCR™ procedure was used to generate Cry1C R148D mutants containing amino acid substitutions in loop α5-6, including amino acid positions 178-184. The mutagenic oligonucleotide primers used to generate mutations encoding substitutions in loop α5-6 are listed below.

Position 178: Primer R (SEQ ID NO:70)

5'-GATTCTGTAATTTTTNNAGAAAGATGGGGATTGACAACGATAAATGTCAATG -3'

N (16) = 50% C; 50% G

N (17) = 33.3% C; 33.3% G; 33.3% A

Position 179: Primer S (SEQ ID NO:71)

5'-GATTCTGTAATTTTTGGANNAAGATGGGGATTGACAACGATAAATGTCAATG -3'

N (19) = 50% C; 50% G

N (20) = 33.3% C; 33.3% G; 33.3% A

Position 180: Primer T (SEQ ID NO:72)

5'-GATTCTGTAATTTTTGGAGAANNATGGGGATTGACAACGATAAATGTCAATG -3'

N (22) = 50% C; 50% G

N (23) = 33.3% C; 33.3% G; 33.3% A